Methods: Umbilical cords were dissected to obtain WJ and MC was obtained from a ~2 cm section of whole cord, which was minced into small (~2mm^2) pieces and digested in collagenase I for 1 h at 37°C. Resulting cells were cultured in DMEM-F12, 10 % FCS and Penicillin/streptomycin. Flow cytometry, immunocytochemistry and Western blotting was used to characterise cell markers indicative of pluripotency (SOX2, Nanog, REX-1, OCT3/4, SSEA-3, 4, TRA-1-60, TRA-1-81 and alkaline phosphatase) and immunogenicity (using co-stimulatory markers, CD40, 80, 86, in addition to HLA-G, Indoleamine 2 3-dioxygenase and HLA-DR) before and after stimulation with IFN-γ (500 ng/ml for 48 h). BMSC and UCMSC were cultured with allogeneic CD4$^+$ T cells (responder cells) (at a ratio of 1:5) labelled with violet proliferation dye in addition to peripheral blood mononuclear cells (PBMC) (used as stimulator cells) for 5 days. Controls were CD4$^+$ T cells and PBMCs alone. After 5 days T cells were analysed via flow cytometry to assess their proliferative response.
Results: All cells showed positivity for the markers SOX2, Nanog, REX-1, SSEA-4, Alkaline phosphatase, TRA-1-81, HLA-G and were negative for OCT 3/4, SSEA-3 and the co-stimulatory markers CD40, CD80, CD86. BMSC were positive for TRA-1-60, whereas UC MSC were negative. Differences were seen between BMSC and UC MSC after stimulation with IFN-γ. UC MSC remained negative for the co-stimulatory markers CD40, 80, 86 and HLA-DR after IFN-γ stimulation, but, BMSC showed up-regulation of HLA-DR after exposure to IFN-γ. All cells were negative for IDO before treatment with IFN-γ and became positive after stimulation. T cell proliferation was found to be supressed in all cultures containing either BMSCs or UC MSCs, showing that cells from both of these sources have immunomodulatory properties and may be capable of dampening down immune response *in vivo*.
Conclusions: Umbilical cord derived MSCs may have more promise than BMSCs as an allogeneic cell therapy as they do not produce co-stimulatory markers or MHC (major histocompatibility complex) class II antigens after culture *in vitro* in an inflammatory environment such as that which may be found in an osteoarthritic joint.

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THE USE OF A BI-DIRECTIONAL PERFUSION BIOREACTOR FOR CARTILAGE ENGINEERING PROMOTES THE RECONSTRUCTION OF HYALINE CARTILAGE

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Purpose: We previously reported that a cocktail of bone morphogenetic protein (BMP)-2, insulin and triiodothyronine (BIT) could trigger redifferentiation of human chondrocytes after their amplification on plastic, with cartilage-characteristic matrix reconstruction when the chondrocytes were seeded in collagen sponges (Claus et al., 2011). However, this matrix was not homogenously distributed in the scaffolds, most likely because the collagen sponges were cultivated in static conditions. With the aim of enhancing cellular access to nutrients and the soluble factors, bi-directional flow was tested to perfuse the scaffolds during cartilage reconstruction.
Methods: After 3 weeks of amplification on plastic, HAC were seeded then cultivated for 21 days in collagen sponges under bi-directional flow, by using a prototype of OPB (Oscillating Perfusion Bioreactor). We established a program of perfusion including phases of high and low perfusion speeds to alternate sequences of cell stimulation and matrix deposition. For comparison, cultures of HAC in collagen sponges were performed in static conditions. The status of the chondrocyte phenotype and the nature of the matrix synthesized in collagen sponges were evaluated by real time PCR, Western Blotting and immunohistochemistry analyses. The viability and cell proliferation were also monitored.
Results: The results clearly indicate that perfusion improves cartilage matrix deposition within the sponges, in comparison with static conditions. More precisely, in the sponges cultured in the bioreactor, redifferentiated and metabolically active HAC produced a cartilaginous matrix rich in type II and type IX collagens and in glycosaminoglycans, with no sign of hypertrophy. Interestingly, a much lower amount of type I collagen was produced in the sponges cultivated in dynamic conditions, indicating therefore that bi-directional perfusion limits the risk of fibrocartilage formation.
Conclusions: The combination of HAC, collagen sponges, and the BIT cocktail with the bi-directional perfusion bioreactor favors the reconstruction of hyaline cartilage. Importantly, bi-directional perfusion abolishes the spatial concentration gradients routinely observed in scaffolds in static culture. This study also demonstrates the value of a multi-factorial approach for the design of cell-based grafts for cartilage repair.

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PLATELET-RICH PLASMA TO STIMULATE CARTILAGE HEALING, WHICH PRODUCT? A COMPARATIVE IN VITRO STUDY

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Purpose: Platelet Rich Plasma (PRP), a blood-derived product rich in growth factors, is a promising treatment for cartilage defects in Osteoarthritis (OA). Despite its widespread application there is a lack of in deep studies to demonstrate its real efficacy. Aim of the present study was to investigate the effects of two different PRP preparations on chondrocytes from osteoarthritis patients evaluating both the expression and secretion of pro-inflammatory and anabolic markers and the production of some extracellular matrix proteins.
Methods: Blood from ten human volunteers was used to prepare two different formulation of PRP: 1) Platelet Rich Plasma with a low concentration of platelets and leukocytes (P-PRP), 2) Platelet Rich Plasma with a high levels of platelets and leukocytes (L-PRP). Before the experiments, each PRP was evaluated for growth factors and cytokines content. Chodrocytes were obtained from cartilage tissues of patients with OA (Kellgren-Lawrence grade I-II-III) undergone surgery and were isolated by enzymatic procedure. The cells were seeded at a high density and cultured in presence of three different concentrations (5, 10 and 20%) of both P-PRP and L-PRP for 7 days. The in vitro effects of the two different PRPs on chondrocytes were evaluated through the analysis of cell proliferation, matrix production and expression of specific genes.
Results: Our data showed that L-PRP contains higher levels of growth factors and cytochines compared to P-PRP. Both PRPs at all the concentration evaluated stimulated chondrocyte proliferation throughout the culture period analyzed; at day 7 P-PRP induced greater cell growth compared with L-PRP. P-PRP stimulated chondrocyte anabolism as demonstrated by an increased expression of collagen type II and aggrecan, while L-PRP promoted catabolic pathways in which different cytokines are involved. However, L-PRP was able to induce a higher expression of hyaluronic acid synthase-2 and hyaluronan compared to P-PRP.
Conclusions: Our study highlights some effects exerted by two different PRP compositions on cultured human chondrocytes. These effects are reasonably due to the content of platelets, leukocytes, growth factors and molecules which characterize each compound and that act synergically to regulate some biological processes. The identification of the optimal amount and ratio of these blood components could ideally lead to a PRP more suitable for the treatments of cartilage lesions both of traumatic or degenerative origin.

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THE ENHANCEMENT OF HUMAN BONE MARROW DERIVED MESENCHYMAL STEM CELLS (HBMSCS) FOR SCAFFOLD-FREE CARTILAGE-LIKE CELL-SHEETS

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Purpose: We are developing the cell therapy, especially using hBMSCs, for cartilage defect. The result of this therapy would be better if we could get the cells, having more chondrogenic capacity, enough to target the cartilage defect. The cell-sheet would be a good candidate of the applications to get better result. Our colleagues have reported the